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Mutation cases in the paternity tests using 15 autosomal STR markers

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As well known, the mutations of the STR loci revealed in resolving the identification, disputed paternity/mothernity, kinship, etc. cases reduce to some extent the reliability of the results and deliver a certain difficulty in preparation of an accurate expert opinion. Therefore, information on the facts of detection of such allelic variations has great practical importance. In this study among 250 family cases of disputed paternity we found mutated alleles in two cases on FGA, in two cases on D19S433, in one case on D13S317 and in one case on D5S818 locus. In five cases more likely these mutations affected the paternal alleles, in one case the maternal allele. For each case possible mutation formation ways scheme was proposed. Moreover, in one case three-allelic profile on D21S11 locus has been observed indicating three copies of chromosome 21, which supported existing Down's syndrome phenotype.

Keywords: *STR marker, crossingover, insertion, deletion, null-allele, stepwise mutation, gene conversion, strand-slippage replication, paternity testing, maternal meiosis*

INTRODUCTION

The main directions of the use of STR-markers located on autosomal and sexual choromosomes is forensic examination in order to solve identification problems and, most importantly, disputed paternity/maternity, relationship or kinship tests (Botstein et al., 1980; Sprecher et al., 1994; Urquhart et al., 1994, 1995; Wang et al., 1996; Dupy et al., 1997; Dauber et al., 2004; Butler, 2006; Schneider, 2012; Dogan et al., 2014). In previous studies we (Mustafayev et al., 2016, 2017a, 2017b) applied of STR-markers in various areas of practical molecular biology and medicine, population studies. All these studies were based on the high hypervariability of these loci.

Currently for these purposes different sets of commercial markers, for example Promega PowerPlex 16®, Promega Geneprint FFFL® kit, Applied Biosystems SGM Plus® kit, AmpFlSTR® Identifiler® PCR Amplification Kit, AmpFlSTR® Y-filer® PCR Amplification Kit, etc are used. Studies are intensively conducted to assess the suitability of these markers for use in solving of abovementioned problems. This necessity is primarily associated with the detection of mutations in deciding the questions of disputed paternity/maternity at all loci included in these kits (Hammond et al., 1994; Geada et al., 2003; Aşıcıoğlu et al., 2004; Opolska-Bogusz et al., 2006; Zhao et al., 2007; 2015; Deucher et al.., 2010; Dinesh et al., 2013; Zhang et al., 2014, etc.). Such assessment of 18 frequently used loci in conducting maternity/paternity and kinship tests was regularly conducted American Association of Blood Banks (AABB) from 2001 to 2013 (Annual Report Summary..., 2001, 2002, 2003, 2004, 2006, 2008, 2010, 2013; for details see: https://strbase.nist.gov/mutation.htm, table 1).

As it is known mutations arise mainly in meiosis, i.e. more precisely it occur in pachytene (pachyneme) phase of prophase I (the final phase of genetic recombination or third phase of prophase I) of the meiosis I with the participation of the

synaptoneme complex. In this stage of meiosis occur exchange of genetic material/or information between homologous chromosomes. It should be noted that all types of chromosomal rearrangements – deletions/ insertions, inversions, duplications, translocations, etc., occur in this phase The chance of autosomal chromosome mutations in meiosis is always great compared to sex chromosomes. When the meiotic mutation affects the maternal genetic material it is called maternal meiosis, when paternal genetic material - paternal meiosis.

There are several different approaches for describing mutations in these microsatellite loci which are used in performing of paternity tests (Fan and Chu, 2007). All these points of views theoretically based on stepwise mutation model of T.Ohta and M.Kimura (see: H.Ellegren, 2004). U-D.Immel et al. (2004) showed that the most obvious explanation for a mutation in an STR locus would be a contraction of the repeat stretch due to polymerase slippage and these mutations are almost invariably confined to a single repeat. Based to the experimental data they proposed a gene conversion and DNA crossover model (see: Fig. 3 in Immel et al., 2004). By M.A.Jobling (2004) preferred other – strand-slippage replication mechanism of mutation formation (Fig. 1). It should be noted that larger contractions or expansions are considered extremely rare, and to be the consequence of recombination rather than slippage.

In the literature there is a large amount of data on identified mutations almost for each autosomal STR locus (Boutrand et al., 2001; Leibelt et al., 2003; Ricci et al., 2003; Immela et al., 2004; Edwards and Allen, 2004a, 2004b; Singh et al., 2006; Huel et al., 2007; Narkuti et al., 2007, 2008; Balloch et al., 2008; Natsuko et al., 2008; Eunus et al., 2009; Venkanna et al., 2009; Burkhard et al., 2011; Li et al., 2014; Liu et al., 2015; Cabezas et al., 2016, etc.). For example, in study performed by H.Geada et al. (2003) 511 paternity cases were investigated using SGM Plus and PowerPlex 16 Kits, 18 paternal and maternal mutation cases have been detected in 13 STR loci and types of mutations (one-step, two-step and null allele-mutations) were indicated.

In another sudy of paternity testing by K.J.D.Balloch et al. (2008) 2 exclusions are encountered at D8S1179 and CSF1PO STR loci has been reported. Paternity test repeated using PowerPlex16®, FFFL System® and SGM Plus® kits and detected exclusions were observed. Interestingly, the alleles observed at CSF1PO: alleged father (11, 12), child (10, 11), mother (11, 12) arose as a result of a mutation from either the mother or father. Paternity has been proven Y-chromosome testing using PowerPlex Y® kit which result showed that Y-chromosome haplotypes of alleged father and child are matched.

Табле 1. Apparent mutations observed at STR loci in the course of paternity testing (https://strbase.nist.gov/mutation.htm).

STR System	Maternal Meioses (%)	Paternal Meioses (%)	Number from either	Total Number of Mutations	Mutation Rate
CSF1PO	95/304,307 (0.03)	982/643,118 (0.15)	410	1,487/947,425	0.16%
FGA	205/408,230 (0.05)	2,210/692,776 (0.32)	710	3,125/1,101,006	0.28%
TH01	31/327,172 (0.009)	41/452,382 (0.009)	28	100/779,554	0.01%
TPOX	18/400,061 (0.004)	54/457,420 (0.012)	28	100/857,481	0.01%
VWA	184/564,398 (0.03)	1,482/873,547 (0.17)	814	2,480/1,437,945	0.17%
D3S1358	60/405,452 (0.015)	713/558,836 (0.13)	379	1,152/964,288	0.12%
D5S818	111/451,736 (0.025)	763/655,603 (0.12)	385	1,259/1,107,339	0.11%
D7S820	59/440,562 (0.013)	745/644,743 (0.12)	285	1,089/1,085,305	0.10%
D8S1179	96/409,869 (0.02)	779/489,968 (0.16)	364	1,239/899,837	0.14%
D13S317	192/482,136 (0.04)	881/621,146 (0.14)	485	1,558/1,103,282	0.14%
D16S539	129/467,774 (0.03)	540/494,465 (0.11)	372	1,041/962,239	0.11%
D18S51	186/296,244 (0.06)	1,094/494,098 (0.22)	466	1,746/790,342	0.22%
D21S11	464/435,388 (0.11)	772/526,708 (0.15)	580	1,816/962,096	0.19%
Penta D	12/18,701 (0.06)	21/22,501 (0.09)	24	57/41,202	0.14%
Penta E	29/44,311 (0.065)	75/55,719 (0.135)	59	163/100,030	0.16%
D2S1338	15/72,830 (0.021)	157/152,310 (0.10)	90	262/225,140	0.12%
D19S433	38/70,001 (0.05)	78/103,489 (0.075)	71	187/173,490	0.11%
SE33 (ACTBP2)	0/330 (<0.30)	330/51,610 (0.64)	None reported	330/51,940	0.64%

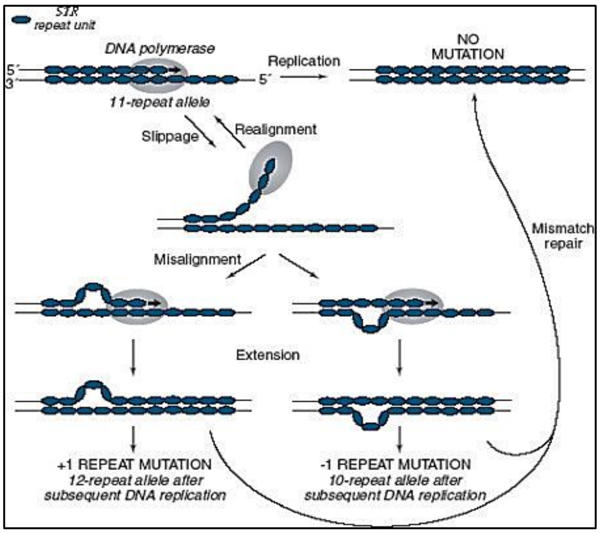


Fig. 1. Schematic illustration of the strand-slippage replication at STR (M.A.Jobling, 2004).

The mutation case in paternity test – two repeat decrease at FGA locus was reported in Turkish family (Canturk et al., 2015). Because of mother and alleged father are homozygous (25, 25) at this loci, but child is heterozygous (23, 25)) the authors failed to establish the source of the reduced allele.

During a population study of 128 Korean families (626 persons) with the AmpF*l*STR Profiler Plus PCR amplification system by G-R.Han et al. (2001) was found an unusual homozygous genotype at the D8S1179 locus in 4 families. To evaluate the cause newly designed primers designed for the D8S1179 locus from GenBank data (GenBank Accession No. G08710) amplified alleles that were not amplified with the AmpF*l*STR Profiler Plus PCR amplification system. Authors sequenced alleles of the family members who had non-amplified alleles and found the point mutation - a G-to-A transition at the position of the 147th base of the GenBank sequence.

In three paternity tests performed by U.Ricci et al. (2003) with a set of autosomal STRs discovered three separate incompatibilities for the loci D3S1358, D8S1179 and D18S51 which was probably due to mutation events. Since in these three cases disputed children were males the paternity of the alleged fathers was confirmed with set of 10 Y-STR markers.

As can be seen from the analysis of the above cited literature data, the study of mutations at the practical application of STR-markers have a great importance. Considering this factor we decided to provide information about the mutational cases revealed at the disputed paternity tests using 15 autosomal STR markers including in the AmpF*l*STR®Identifiler®Plus PCR Amplification Kit during 2012-2018 years, classify them and give a brief analysis of each case.

MATERIAL AND METHODS

Blood samples: Blood samples were collected for paternity tests in period of 2012-2018 (250 tests) from citizens living in territory of Azerbaijan Republic and whose ancestores presumably are Azerbaijan residents for several generations. All blood samples were collected in accordance with international ethics rules: signed consent from all donors collected and further anonymity of individuals were provided. All the chromosomal DNA samples isolated from these blood samples were used only for research purposes based on decision of local ethical committees of both institutions only for research purposes.

DNA samples: DNA were extracted from liquid or dried blood samples using PrepFiler ®DNA Extraction Kit (Applied Biosystems, Life Technologies, USA) according manufacturer's instuctions. Concentrations and purity DNA simples were determined using Quantifiler®Human DNA Quantification Kit in 7500 RealTime PCR System (Applied Biosystems, USA).

Multiple PCR amplification and genotyping: Multiple amplification reactions (total reaction volume is 25 μl) using 0.1 ng genomic DNA were carried out in an ABI 9700 PCR system (Thermo Fisher Scientific Company, USA) for all 15 STR loci (D8S1179, D21S11, D7S820, CSF1PO, D3S1358, THO1, D13S317, D16S539, D2S1338, D18S51, D19S433, vWA, TPOX, D18S51, D5S818 and FGA) included in the AmpF*l*STR®Identifiler®Plus PCR Amplification Kit (Identifiler_V2). After PCR amplification the reaction products were denatured with formamide (Hi-Di) and internal size standard GeneScan ™-500 LIZ®SizeStandard (Thermo Fisher Scientific Company, USA). Electrophoresis of all amplified PCR products and genotype profiling were performed using HITACHI ABI 3130 DNA Genetic Analyzer (Applied Biosystems, USA) and GeneMapper ID software v. 3.2 (Applied Bio-systems, USA) respectively. All PCR amplification, denatu-

ration, electrophoresis and gene profiling experimental procedures were carried out according to the manufacturer's guidelines (AmpF*l*STR® Identifiler®Direct PCR Amplification Kit User Guide, 2015). At performance of the work were met all conditions of accuracy and taken into account the DNA Commission of the International Society for Forensic Genetics (ISFG) recommendations (Prinz et al., 2007; Genetic diversity analysis…, 2003; Gusmao et al., 2006; Schneider, 2007, 2012).

RESULTS AND DISCUSSION

It is known that mutations in STR loci occur both in maternal and paternal meiosis. But, the mutation rate for paternal meiosis is higher than maternal meisosis (see: Annual Reports…, 2001-2013). It should be noted that the chance (probability) of one-step mutations is much higher than 2, 3- and multi-sttep mutations. For example, Brinkmann et al. (1998) reported that in 10,844 parent/child allelic transfers at nine STR loci 23 isolated STR mismatches observed and of 23 STR mutations found, 22 were by a single step; one by a double step. C.H.Brenner (2009) based on numerous literary reports, suggested that there is no common formula for description of the of STR marker mutation cases, therefore it can be assumed that:

- 50% of all mutations increase by one step;
- 50% decrease by one step;
- 5% increase by two steps;
- 5% decrease by two steps;
- 0.5% increase by three steps;
- 0.5% decrease by three steps;
- ... etc.

We observed 6 mutation events during paternity tests in period of 2012-2018 (~250 tests). These cases have not been proven either directly or indirectly (for example, isolating and sequencing a mutated allele, involvement of additional autosomal markers, using markers of X- or Y-chromosomes etc.). Therefore, we present the DNA profiles of each case for persuasiveness. Below we will try to discuss and interpret each case separately according to the existing literature data, generally accepted models and mechanisms of formation of such mutations.

2 cases of mutation at the FGA locus were found (Fig. 2a and 2b, table 2). The FGA loci have complex tetranucleotide repeat: $[TTTC]_3TTTTTTCT[CTTT]_nCTCC[TTCC]_2$. Total mutation rate is 0.28%.

In the first case assuming that the maternal allele is not mutated (i.e. Child1 inherited allele **21** from mother), then based on the literature data (Ali et al., 2009), the more likely scheme of mutation formation can be represented as multi-step mutation process: (1) Ch1(17)=AF1(20) – 3 repeat or Ch1(17)=AF1(23) – 6 repeat (hereinafter Ch-child, AF – alleged father, AM – alleged mother). First scheme is more reasonable. Note that it is not excluded and such a mutation scheme, according to which the mutation affects both the maternal and the paternal alleles simultaneously, i.e. occurs double one-step mutation – one with reducing allele size (deletion), another with increasing allele size (insertion), for example: Ch1(17)=AM1(18) – 1 repeat and Ch1(21)=AF(20) + 1 repeat.

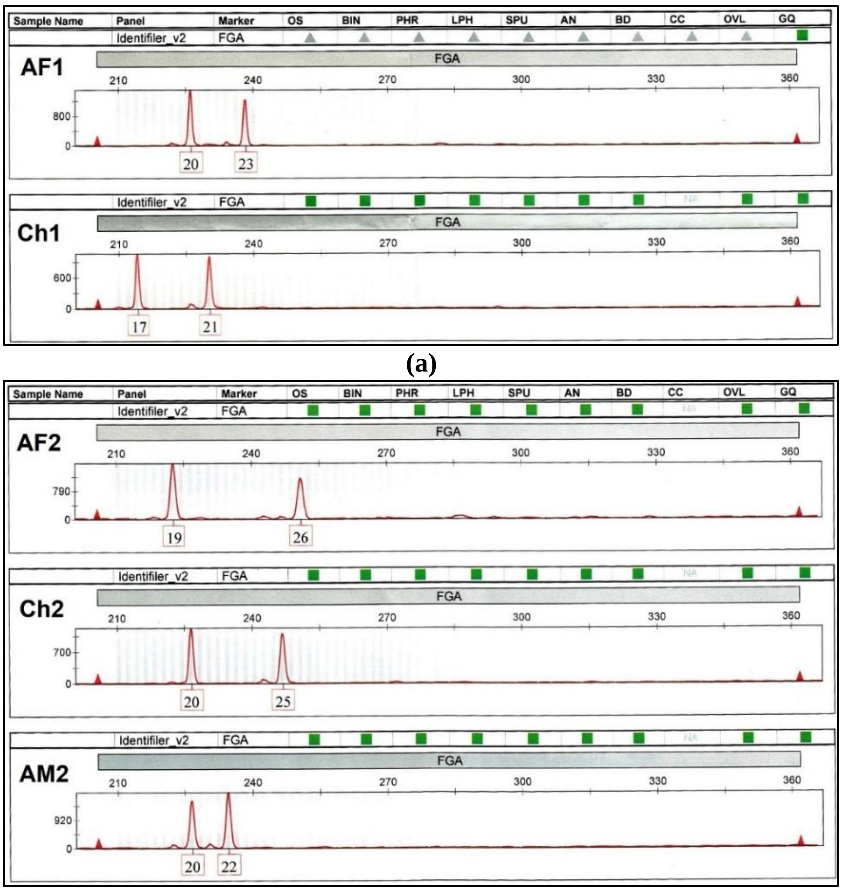
Below the DNA sequences of the all alleles that are present in the DNA profiles at the FGA locus of the tested individuals are shown.

Allele	Sequence	Owner
17	$[TTTC]_3$TTTT TTCT$[CTTT]_9$CTCC$[TTCC]_2$	**Ch1**
18	$[TTTC]_3$TTTT TTCT$[CTTT]_{10}$CTCC$[TTCC]_2$	**AM1**
20	$[TTTC]_3$TTTT TTCT$[CTTT]_{12}$CTCC$[TTCC]_2$	**AF1**
21	$[TTTC]_3$TTTT TTCT$[CTTT]_{13}$CTCC$[TTCC]_2$	**AM1/ Ch1**
23	$[TTTC]_3$TTTT TTCT$[CTTT]_{15}$CTCC$[TTCC]_2$	**AF1**

Analogically, the second case of mutation at this locus can be explained more simply: Ch2(25)=AF2(26) – 1 repeat (one-step mutation).

During paternity testing another 2 cases of mutation were detected at the D19S433 locus (Fig. 3a and b, table 3). The locus have repeat structure: $(AAGG)(AAAG)(AAGG)(TAGG)[AAGG]_n$. Total mutation rate for this loci is 0.11%. First mutational event is paternal allele mismatch and second event is maternal allele mismatch. For the first case more reasonable mutation scheme can be presented as a deletion: Ch1(15.2)=AF1(16.2) – 1 repeat (one-step mutation "del" type).

Second mutational case on D19S433 locus represent maternal allele mutation with more complex character. Mother (AM2) and child (Ch2) on this locus are homozygous. One way of mutation can be represented as insertion of 2 repeats and 2 nucleotides: Ch2(15.2)=AM2(13) + 2 repeat+2 b.p. or as insertion of 3 repeats and subsequential loss of 2 nucleotides: Ch2(15.2)=AM2(13) + 3 repeat – 2 b.p. But the probability of such conversion schemes is very low.



(a)

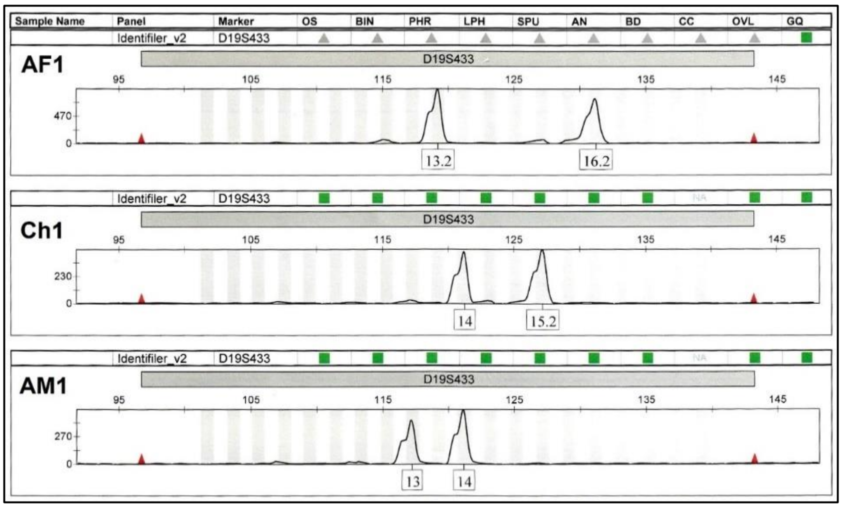
(b)

Fig. 2. The mutation cases observed on FGA locus in the form of paternal allele mismatch. Hereinafter: AM – alleged mother; Ch – child; AF – alleged father. Note: in case (a) since the data on mother DNA profiles are presented to us by the applicants, therefore the picture of the DNA profile is missing.

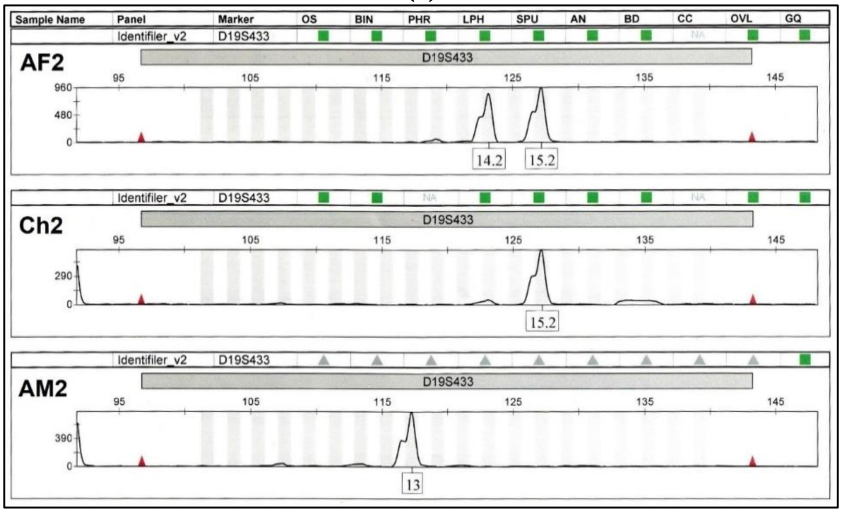
Table 2. The mutation cases at the FGA locus observed in two paternity tests.

No	STR loci	Family 1				Family 2			
		***AM1**	**Ch1**	**AF1**	**Status**	**AM2**	**Ch2**	**AF2**	**Status**
1	D8S1179	14, 16	14, 16	13, 14	match	13, 14	13, 15	15, 15	match
2	D21S11	30, 31.2	31.2, 31.2	30.2, 31.2	match	31.2, 31.2	31.2, 32.2	31.2, 32.2	match
3	D7S820	11, 11	10, 11	9, 10	match	11, 13	11, 13	8, 13	match
4	CSF1PO	10, 11	11, 11	10, 11	match	12, 13	12, 12	12, 12	matsch
5	D3S1358	15, 19	15, 17	17, 17	match	16, 17	17, 17	17, 17	match
6	THO1	8, 9.3	6, 8	6, 8	match	6, 8	8, 9	9, 9	match
7	D13S317	12, 12	11, 12	11, 12	match	10, 11	10, 11	11, 11	match
8	D16S539	11, 13	11, 11	11, 13	match	10, 12	10, 10	9, 10	match
9	D2S1338	17, 20	17, 25	22, 25	match	17, 25	17, 25	17, 20	match
10	D19S433	14.2, 15.2	13, 15.2	12, 13	match	14, 14	14, 15	15, 16.2	match
11	vWA	16, 17	14, 17	14, 15	match	15, 15	15, 18	14, 18	match
12	TPOX	8, 11	8, 11	8, 11	match	10, 12	10, 12	8, 10	match
13	D18S51	12, 14	12, 15	12, 15	match	13, 16	13, 16	13, 13	match
14	D5S818	11, 13	11, 11	11, 11	match	11, 12	11, 11	9, 11	match
15	FGA	18, **21**	17, **21**	20, 23	**mismatch**	**20**, 22	**20**, 25	19, 26	**mismatch**
16	Amelogenin	X, X	X, X	X, Y		X, X	X, Y	X, Y	

***Notes:* Hereinafter:** AM – alleged mother; Ch – child; AF – alleged father; Possible parental alleles marked in bold, mutated alleles indicated in box; * - DNA profiles of the mother (AM1) were presented to us by the aplicants on the disputed paternity test.



(a)



(b)

Fig. 3. The mutation cases observed on D19S433 locus: (a) paternal allele mismatch; (b) maternal allele mismatch.

Table 3. The mutation cases at the D19S433 locus observed in two paternity tests.

No	STR loci	Family 1				Family 2			
		AM1	Ch1	AF1	Status	AM2	Ch2	AF2	Status
1	D8S1179	11, 15	15, 17	11, 17	match	10, 12	12, 14	11, 14	match
2	D21S11	27, 30	27, 30	30, 31.2	matsch	29, 31	29, 29	29, 32	matsch
3	D7S820	10, 12	10, 12	10, 11	match	8, 12	8, 11	11, 12	match
4	CSF1PO	12, 13	10, 12	10, 11	match	9, 12	10, 12	10, 10	match
5	D3S1358	14, 15	15, 16	16, 17	match	15, 17	17, 18	15, 18	match
6	THO1	6, 9	9, 9.3	6, 9.3	match	8, 9	9, 9.3	6, 9.3	match
7	D13S317	8, 13	8, 12	12, 12	match	11, 12	9, 11	9, 11	match
8	D16S539	11, 12	11, 12	12, 13	match	12, 12	11, 12	11, 12	match
9	D2S1338	21, 23	17, 23	17, 17	match	18, 25	22, 25	17, 22	match
10	D19S433	13, **14**	**14**, 15.2	13.2, 16.2	**mismatch**	13, 13	15.2, **15.2**	14.2, **15.2**	**mismatch**
11	vWA	17, 19	15, 17	15, 15	match	15, 19	15, 19	15, 16	match
12	TPOX	8, 11	11, 11	8, 11	match	11, 11	8, 11	8, 8	match
13	D18S51	14, 16	12, 14	12, 14	match	16, 18	15, 16	15, 15	match
14	D5S818	11, 15	12, 15	12, 12	match	13, 13	11, 13	11, 12	match
15	FGA	21, 21	21, 24	22, 24	match	21, 23	21, 24	21, 24	match
16	Amelogenin	X, X	X, Y	X, Y		X, X	X, Y	X, Y	

Table 4. The mutation case at the D13S317 locus observed in paternity tests.

No	STR loci	AM	AF	Ch1	Status	Ch2	Status
1	D8S1179	15, 15	14, 14	14, 15	match	14, 15	match
2	D21S11	29, 33.2	31.2, 31.2	31.2, 33.2	match	31.2, 33.2	match
3	D7S820	10, 11	8, 10	10, 10	match	8, 10	match
4	CSF1PO	10, 10	11, 11	10, 11	match	10, 11	match
5	D3S1358	15, 18	16, 18	15, 18	matsch	18, 18	match
6	THO1	7, 9.3	7, 9.3	9.3, 9.3	match	7, 7	match
7	D13S317	**10**, 11	**8**, 13	**8**, **10**	match	**10**, 12	mismatch
8	D16S539	11, 12	11, 12	11, 12	match	11, 11	match
9	D2S1338	20, 24	17, 18	17, 20	match	18, 20	match
10	D19S433	15, 15	14, 15	14, 15	match	15, 15	match
11	vWA	14, 16	16, 17	16, 17	match	14, 16	match
12	TPOX	9, 11	8, 11	9, 11	match	8, 11	match
13	D18S51	15, 16	12, 12	12, 16	match	12, 16	match
14	D5S818	12, 13	9, 12	12, 13	match	9, 12	match
15	FGA	19, 25	20, 23	19, 20	match	20, 25	match
16	Amelogenin	X, X	X, Y	X, Y		X, X	

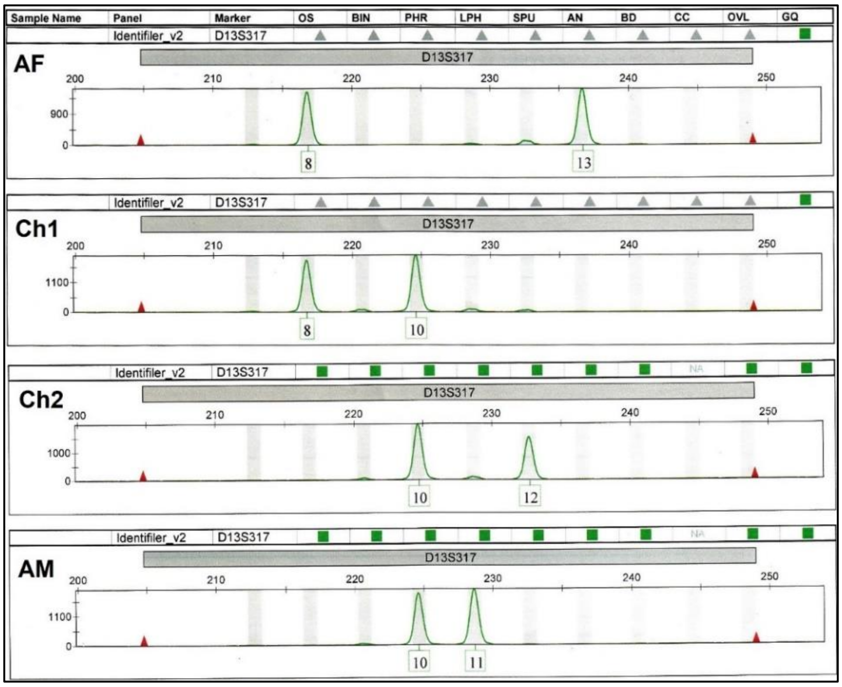


Fig. 4. The mutation case observed on D13S317 locus – paternal allele mismatch in second child.

However, the point mutations (basically in the form of nucleotide transitions or transversions) in flanking regions of locus, i.e. in primer-binding sites, that leads to non-amplification and lost of maternal allele, in other words probability of conversion of maternal allele to the null-allele also is very high. It should be noted that allele frequencies of 14.2 and 16.2 (0.0248 and 0.0149 respectively) are much less than allele 15.2 (0.1225) for our population (Mustafayev et al., 2017b).

Allele	Sequence	Owner
13	(AAGG)(AAAG)(AAGG)(TAGG)(**AAGG**)$_{11}$	**AM1/ AM2**
14	(AAGG)(AAAG)(AAGG)(TAGG)(**AAGG**)$_{12}$	**AM1/ Ch1**
14.2	Sequence no available	**AF2**
15.2	Sequence no available	**Ch1/ Ch2/ AF2**
16.2	Sequence no avaliable	**AF1**

Next mutation event is observed at the D13S317 locus (Fig. 4, table 4). Total mutation rate for this loci is 0.14%. The locus have repeat structure [**GATA**] (bottom strand (commonly used)) or [**TATC**] (GenBank top strand). As it is seen from the table 4, the mutation affected only the second child allele. More reasonable mutation scheme can be represented as one-step mutation with deletion leading to reduction of paternal allele size: Ch1(12)=AM(13) – 1 repeat. Below the DNA sequences of the all alleles that are present in the DNA profiles of the tested individuals are shown.

Allele	Sequence	Owner
8	**(TATC)**$_8$	**AF/Ch1**
10	**(TATC)**$_{10}$	**AM/Ch1/Ch2**
11	**(TATC)**$_{11}$	**AM**
12	**(TATC)**$_{12}$	**Ch2**
13	**(TATC)**$_{13}$	**AF**

Another mutation case was detected on D5S818 locus (table 5, Fig. 5). Total mutation rate on this locus is 0.11% (0.025% during maternal and 0.12% paternal meiotic stages). The locus repeat structure is [**AGAT**]$_n$ (GeneBank top strand).

If it is assumed that allele 14 in the child's DNA profile is inherited from the mother, then the presence of allele 12 can be explained as follows: (1) as a result of one-step mutagenesis with the addition of one repeat as Ch(12)=AF(11) + 1 repeat (allele size extension) and (2) as a result of one-step mutagenesis with the loss of one repeat as Ch(12)=AF(13) – 1 repeat (allele size reduction). For clarity, below the DNA sequences of all alleles present on the DNA profiles of the mother, child and alleged father are presented.

Allele	Sequence	Owner
10	**(AGAT)**$_{10}$	**AM**
11	**(AGAT)**$_{11}$	**AF**
12	**(AGAT)**$_{12}$	**Ch**
13	**(AGAT)**$_{13}$	**AF**
14	**(AGAT)**$_{14}$	**AM/Ch**

Table 5. The mutation case at the D5S818 locus observed in paternity tests..

No	STR loci	AM	Ch	AF	Status
1	D8S1179	12, 14	14, 14	14, 14	match
2	D21S11	30, 30	30, 33.2	31, 33.2	match
3	D7S820	10, 11	10, 11	10, 11	match
4	CSF1PO	10, 12	12, 13	12, 13	match
5	D3S1358	16, 16	15, 16	13, 15	match
6	THO1	6, 9.3	6, 9.3	6, 7	match
7	D13S317	8, 11	8, 11	8, 8	match
8	D16S539	9, 13	9, 9	9, 12	match
9	D2S1338	17, 20	17, 25	20, 25	match
10	D19S433	12, 13	12, 14.2	13, 14.2	match
11	vWA	16, 19	19, 19	17, 19	match
12	TPOX	8, 8	8, 8	8, 8	match
13	D18S51	15, 17	17, 21	16, 21	match
14	D5S818	10, **14**	12, **14**	11, 13	mismatch
15	FGA	23, 23	23, 23	23, 23	match
16	Amelogenin	X, X	X, X	X, Y	---

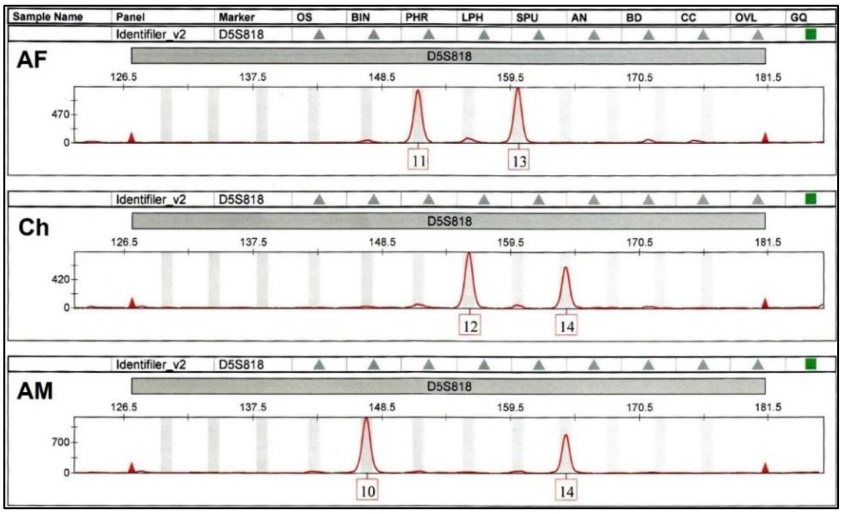


Fig. 5. The mutation case observed on D5S818 locus – paternal allele mismatch.

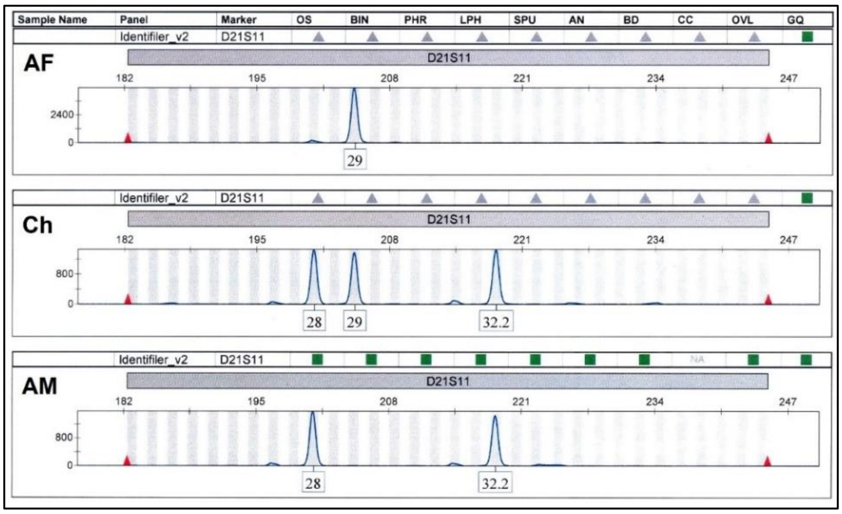


Fig. 6. The mutation case observed on D21S11 locus – trialleic variant indicating Downs syndrome.

Most interesting mutation case observed by us is trialleic variant revealed in the child's DNA profile on the D21S11 locus (Fig. 6, table 6). This indicated the chromosome 21 trisomy, which was in agreement with existing Down's syndrome phenotype. Since the father was homozygous for allele 29 and mother had alleles 28 and 32.2 in D21S11 STR locus, we assumed that child inherited extra chromosome 21 from the mother.

Table 6. The triallelic mutation case at the D21S11 locus observed in paternity tests.

No	STR loci	AM	Ch	AF	Status
1	D8S1179	12, 13	12, 14	14, 15	match
2	D21S11	**28, 32.2**	**28, 29, 32.2**	**29, 29**	**match (trialleic variant)**
3	D7S820	10, 11	7, 11	7, 9	match
4	CSF1PO	12, 12	12, 12	10, 12	match
5	D3S1358	16, 18	16, 16	16, 18	match
6	THO1	6, 9.3	6, 9.3	6, 9	match
7	D13S317	9, 10	10, 12	11, 12	match
8	D16S539	12, 12	10, 12	9, 10	match
9	D2S1338	20, 23	20, 23	22, 23	match
10	D19S433	12, 13	13, 14	14, 15	match
11	vWA	16, 19	16, 16	16, 17	match
12	TPOX	8, 11	11, 11	8, 11	match
13	D18S51	11, 15	14, 15	13, 14	match
14	D5S818	9, 12	12, 12	11, 12	match
15	FGA	21, 22	19, 21	19, 27	match
16	Amelogenin	X, X	X, X	X, Y	---

Patterson et al. (2009) described in detail the Down syndrome arising mechanisms and its different forms. Basically the trisomy of 21st chromosome is caused by a failure of the this chromosome to separate during egg or sperm development. Due to the chromosome nondisjunction a sperm or egg cell have an extra copy of 21st chromosome, therefore when this cell combined with a normal cell from the other parent, the baby has 47 chromosomes. It should be noted that about 88% of cases of the 21st chromosome trisomy happens due to nondisjunction of the maternal, 8% cases due to nondisjunction of the paternal chromosomes, and in 3% cases after the egg and sperm have merged.

CONCLUSIONS

As well known, the allele variants due to mutations of the STR loci revealed during resolving the identification, disputed paternity/mothernity, kinship, etc. problems to some extent reduce the reliability of the results and deliver a certain difficulty in preparation of an accurate expert opinion. Therefore, information regarding the facts of detection such allelic variations has great importance. Given this, we found it appropriate to inform the scientific community and practicing specialists in this area about the mutations identified by us over a certain period by using of 15 autosomal STR markers including in the AmpF*l*STR® Identifiler®Plus PCR Amplification Kit.

Analyzing the observed mutational cases based on literary data, we can conclude that 5 cases of

them (2 cases on FGA, first case on D19S433, 1 case on D13S317 and 1 case on D5S818 locus) are ins/del type mutations with reducing/increasing of an allele size the occurrence of which can be explained by a step-wise mutation model or strand-slippage replication model. For the one case (the second case on D19S433 locus) a more reasonable explanation is that the point mutation(s) affected the primer binding site which led to maternal allele lost or to conversion it into a null allele. The trialleic variant revealed in the child DNA profile on the D21S11 locus indicates the existence of 21st chromosome trisomy (i.e. Down's syndrome), which can be explained that the existence of extra copy of 21st chromosome the child DNA profile is inherited from the mother. Summarizing the foregoing we assume that our report on detected mutations is important not only in terms of statistics, but will be also off grate value for practicing experts.

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15 Autosom STR marker istifadə edilən atalıq testlərində mutasiya halları

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Məlumdur ki, identifikasiya, mübahisəli atalıq/analıq, qohumluq və s. məsələlərin həlli zamanı STR lokuslarda aşkarlanan mutasiyalar müəyyən dərəcədə nəticələrin etibarlılığını aşağı salır və dəqiq ekspert rəyinin tərtibində müəyyən çətinliklər yaradır. Buna görə də belə allel variantlarının aşkarlanması haqqında məlumatlar mühüm praktiki əhəmiyyət kəsb edir. Bu tədqiqatda bizim tərəfimizdən 250 ailədən ibarət mübahisəli atalıq testlərində iki halda FGA, iki halda D19S433, bir halda D13S317 və bir halda D5S818 STR lokusu üzrə mutant allellər aşkarlanmışdır. Beş halda bu mutasiyaların daha böyük ehtimalla atadan mənimsənilən allellərdə, bir halda isə anadan mənimsənilən alleldə baş verməsi güman edilir. Hər bir hal üçün mutasiyaların yaranma yollarının mümkün sxemləri təklif edilmişdir. Bunlardan başqa bir halda 21-ci xromosomun trisomiyasını göstərən D21S11 STR lokusu üzrə üç allelli profil aşkarlanmışdır ki, bu da real olaraq Daun sindromunu müəyyən edən edən mutasiyanın baş verməsini təsdiq etmişdir.

Açar sözlər: *STR marker, krossinqover, insersiya, delesiya, null-allel, mərhələli mutasiya, genlərin konversiyası, zəncir-sürüşməli replikasiyası, atalıq testi, ana meyozu*

Случаи мутаций в тестах на отцовство с использованием 15 аутосомных STR-маркеров

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Известно, что мутации STR локусов, выявленные при решении задач идентификации, спорного отцовства/материнства, родства и др. приводят в некоторой степени к снижению достоверности результатов, что представляет определенную трудность в составлении точного экспертного заключения. Поэтому информация о фактах обнаружения таких аллельных вариаций имеет большое практическое значение. В данном исследовании из 250 семейных случаев спорного отцовства, нами был выявлен мутантный аллель в двух случаях по локусу FGA, в двух случаях по D19S433, в одном случае по D13S317 и в одном случае по локусу D5S818. В пяти случаях более вероятно, что эти мутации затронули отцовские аллели, в одном случае - материнский аллель. Для каждого случая была предложена схема возможных путей образования мутаций. Более того, в одном случае был обнаружен трехаллельный профиль по локусу D21S11, что указывает на трисомию 21-ой хромосомы, что подтверждает существующий фенотип синдрома Дауна.

Ключевы слова: *STR маркер, кроссинговер, инсерция, делеция, нуль-аллель, ступенчатое мутирование, конверсия генов, репликация с проскальзыванием цепи, тест на отцовство, материнский мейоз*